SOLVING THE MYSTERY OF DUAL SALT SYSTEMS AND BINDING CAPACITIES IN HIC

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Motivation

In 2009, Senczuk et al. [1] reported an increase in the dynamic binding capacity (DBC) of a Hydrophobic Interaction Chromatography (HIC) when using dual salt buffer systems. Up to this day, no satisfactory explanation could be found to why and how the presence of two salts would mediate this dramatic increase of the protein binding capacity.

AIM OF STUDIES

To test the influence of surface tension and ionic strength of the buffers used as mobile phase in protein binding.

Dynamic Binding Capacity (DBC) Experiments

The experiments were performed using a Drop Shape Analyzer for surface tension measurements and an ÄKTA pure for the DBC experiments with lysozyme and a monoclonal antibody (mAb) as the loading samples.

1. Influence of Surface Tension in Protein Binding Capacity

2. Influence of Ionic Strength in Protein Binding Capacity

The capacities for the different buffers got much closer which is even more evidenced in the case of the mAb than for lysozyme. IONIC STRENGTH HAS INFLUENCE IN THE DBC.

The salt with the lowest molar surface tension increment accumulates in the surface of the drop which could lead to a shrinkage of the hydration shell of the protein and, hence, higher DBC. [2]

New buffers were tested and a 2M acetate buffer with same ionic strength as the others achieved the highest values of DBC. [2]

Conclusions

1. The surface tension is not the driving force for protein binding.

Different dual salt systems, sharing similar ionic strength can lead to similar binding capacities, concluding that ionic strength is the driving force for protein binding.

2. One salt buffers with similar ionic strength to dual salt systems can promote similar DBCs, contradicting the previously mentioned [1].

References


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